Obtaining lactic acid through microbial fermentation from corn residues

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Obtención de ácido láctico mediante fermentación microbiana a partir de residuos de maíz

Obtenció d'àcid làctic mitjançant fermentació microbiana a partir de residus de blat de moro

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ABSTRACT

The production and kinetics of lactic acid (LA) by batch fermentation were studied, using corn residues as substrate with Lactobacillus delbrueckii spp. Bulgaricus. A factorial design was developed for the pretreatment stage of the residue and lactic fermentation to determine the best conditions for obtaining total reducing sugars and lactic acid. In the pretreatment, the best conditions were obtained by working with HCl at 3% (v/v), 75 °C, and 30 minutes of reaction. Fermentation was carried out, keeping the medium at a pH between 5.5-6, and the best working conditions were 100 µl of the microorganism, 37°C, and 24 hours of fermentation, reaching a maximum concentration of 19.9 g/L. Kinetic models for LA production, bacterial growth, and substrate consumption were proposed. Likewise, to verify the observed data, different statistical indices were calculated to evaluate the accuracy of the models. Finally the Luedeking-Piret and logistic models, were selected as they presented the best fit to the experimental data for LA production, substrate consumption, and bacterial growth, respectively.

Keywords: Lactic acid, lactic fermentation, kinetics model, corn stover.

RESUMEN

Se estudió la producción y cinética de ácido láctico (LA) por fermentación discontinua, utilizando como sustrato residuos de maíz con Lactobacillus delbrueckii spp. Bulgaricus. Se desarrolló un diseño factorial para la etapa de pretratamiento del residuo y fermentación láctica, para determinar las mejores condiciones de obtención de azúcares reductores totales y ácido láctico. En el pretratamiento las mejores condiciones se obtuvieron al trabajar con HCl al 3% (v/v), 75 °C y 30 minutos de reacción. La fermentación se llevó a cabo manteniendo el medio con un pH entre 5.5- 6 y las mejores condiciones de trabajo fueron 100 µl del microorganismo, 37 °C y 24 h de fermentación, alcanzando una concentración máxima de 19.9 g/L. Se plantearon modelos cinéticos para la producción de LA, crecimiento de la bacteria y consumo de sustrato. Asimismo, para constatar los datos observados se calcularon diferentes índices estadísticos para evaluar la precisión de los modelos. Finalmente se seleccionaron los modelos de Luedeking-Piret y logístico, por ser quienes presentaron mejor ajuste a los datos experimentales para la producción de LA, consumo de sustrato y crecimiento bacteriano respectivamente.

Palabras claves: Ácido láctico, fermentación láctica, modelo cinético, rastrojo de maíz.

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RESUM:

Es va estudiar la producció i cinètica d'àcid làctic (LA) per fermentació discontínua, utilitzant com a substrat residus de blat de moro amb Lactobacillus delbrueckii spp. Bulgaricus. Es va desenvolupar un disseny factorial per a l'etapa de pretractament del residu i la fermentació làctica, per determinar les millors condicions d'obtenció de sucres reductors totals i àcid làctic. Al pretractament les millors condicions es van obtenir en treballar amb HCl al 3% (v/v), 75 °C i 30 minuts de reacció. La fermentació es va dur a terme mantenint el medi amb un pH entre 5.5-6 i les millors condicions de treball van ser 100 µl del microorganisme, 37 °C i 24 h de fermentació, aconseguint una concentració màxima de 19.9 g/L. Es van plantejar models cinètics per a la producció de LA, creixement del bacteri i consum de substrat. Així mateix, per constatar les dades observades es van calcular diferents índexs estadístics per avaluar la precisió dels models. Finalment es van seleccionar els models de Luedeking-Piret i logístic, per ser els que van presentar millor ajustament a les dades experimentals per a la producció de LA, consum de substrat i creixement bacterià respectivament.

Paraules clau: Àcid làctic, fermentació làctica, model cinètic, rostoll de blat de moro.

INTRODUCTION

Because of the environmental concern caused by waste management problems, the scarcity of fossil fuels, the emission of greenhouse gases, and the sustainable recovery of products, there is a growing interest in converting biomass of agro-industrial origin into final products[1,2]. Biomass, especially lignocellulosic, is used as a promising substrate given its wide application in obtaining chemical products[3].

Corn stover is abundant lignocellulosic biomass in Ecuador, generated as crop residue in one of the most important agricultural items in the country. Had been reported that 53% of producers in the region use it to feed their livestock, 27% use it for top dressing, leaving it on the ground, and the remaining 20% are burned[4]. This residue contains 35% cellulose, 20% hemicellulose, and 12% lignin[5]. Given its composition, it can be used as a substrate in fermentation processes to obtain different biomolecules, one of them being lactic acid[6].

Lactic acid (LA) is a natural organic acid discovered by Carl Scheele and isolated from sour milk in 1780. It is an important chemical used in the food, pharmaceutical, cosmetic and chemical industries. Other compounds, such as acrylic acid, propylene glycol, and acetaldehyde, are used in obtaining it. It also serves as a precursor to producing biodegradable polymers and green solvents[7,8].

This carboxylic acid has two optical isomers: L (+) and D (-), which can be obtained by chemical synthesis or microbial fermentation[9]. Biotechnology is more attractive and beneficial for the industry since it has the advantages of using renewable sources to reduce the cost of the substrate, in addition to low operating temperatures and pressures, which entails low energy consumption[10,11].

Given the recent development of industrial bioconversion technology, lactic acid is obtained mainly through fermentation by suitable microbial strains under optimal conditions, promoting sustainable production on an industrial scale[12]. For its production, homofermentative lactic acid bacteria (LAB) use is recommended due to its high yield, productivity, and optical purity. On this list is the bacterium Lactobacillus delbrueckii spp. bulgaricus, for being a potential fermenting microorganism for the production of LA[13].

In this sense, corn stover was used as a substrate in lactic acid production through microbial fermentation as it is a widely available resource in Ecuador[14]. The use of waste contributes to environmental problems, and allows its revaluation by using it as a raw material to obtain a product of great applicability in different sectors of the industry.

MATERIALS AND METHODS

Sample collection and preparation

The corn stover was collected in the province of Manabí, Ecuador, during the planting season from October to December 2021. The residue was ground in a rotary crusher and subsequently sieved with an ASTM Nº 70 sieve (mesh 212 µm) to reduce the size of the material and favor hydrolyzate and lactic acid fermentation.

Physical-chemical characterization of the sample

The corn stover characterization was made in terms of moisture content, pH, extractable matter, cellulose, holocellulose, hemicellulose, and lignin. Moisture was determined according to the ASTM-E871 standard[15]. For this, 1 g of sample was taken and placed in an oven at a temperature of 105 + 2 °C for 1 hour. The moisture percentage was calculate by weight difference (1).

$$\%Moisture = \frac{sampleweight(g) - drysampleweight(g)}{sampleweight(g)} * 100\% \quad (1)$$

The pH was measured by direct potentiometry, mixing 9 g of sample in 60 mL of distilled water in a beaker for 1 minute, using a magnetic bar and a stirring plate. Before the pH reading, the electrode bulb was cleaned with distilled water, and after washing, a three-point calibration was performed[16].

The extractable matter was calculated according to the TAPPI T-264 cm-97 standard, as described by Hernández et al.[17], through a Soxhlet extraction process with a mixture of solvents, C_2H_5OH:C_7H_8 (1:2, v:v) for 6 hours. The sample was rinsed with C_2H_5OH and subsequently subjected to a second extraction with C_2H_5OH at 95% (v/v) for 4 hours. In the end, the sample was washed with 500 mL of hot distilled water and dried at room temperature. The percentage of extractable was found with (2).

$$\%Extractable = \frac{sampleweight(g) - extractablefreesampleweight(g)}{sampleweight(g)} * 100\% \quad (2)$$

Cellulose was determined using the Kurschner and Hoffer method used by Hernández et al.[17], for which 1 g of extractable-free sample was weighed and placed in a flask with 20 mL of C_2H_5OH and 5 mL of HNO_3. The mixture was refluxed in a thermostatic bath for 30 minutes, filtered, and the solid was subjected to second digestion for 30 minutes with 25 mL of C_2H_5OH-HNO_3. Subsequently, it was filtered, and the solid sample was subjected to third digestion with 100 mL of distilled water for 1 hour. It was then filtered and washed with hot distilled water, followed by 100 mL of anhydrous $C_2H_3NaO_2$ saturated solution and 500 mL of hot distilled water. Finally, the residue was dried in an oven at 100 °C, kept in a desiccator, and weighed. The cellulose content was found by (3).

$$\%Cellulose = \frac{drysampleweight(g)}{extractablefreesampleweight(g)} * 100\% \quad (3)$$

To determine holocellulose, the Jayme-Wise method, described by Hernández et al.[17], was followed. 5 g of the sample free of extractable were weighed, 1.5 g of sodium chlorite ($NaClO_2$) were added to 160 ml of water, 1 mL of CH_3-COOH and placed in a thermostatic bath at 80°C for 1 hour. Subsequently, 1.5 g of $NaClO_2$ and 1 mL of CH_3-COOH were added, stirred, and allowed to stand for 2 hours. It was washed with 500 mL of hot distilled water and with 250 mL of acetone. The sample was allowed to dry for 12 hours at 70°C to constant weight. It was allowed to cool in a desiccator and weighed, thus determining the holocellulose content.

Lignin was quantified with the Klason Method used by Hernández et al[17]. 0.50 g of extractable-free sample was taken, 15 mL of H_2SO_4 at 72% (v/v) was added, and it was kept under stirring for 2 hours. A 4% (v/v) solution of H_2SO_4 was made by adding 560 mL of distilled water, and it was gently boiled for 4 hours, keeping the volume constant. The sample was cooled to room temperature and subsequently decanted, filtered and placed in an oven at 50 °C for 24 h. Finally, it was weighed, thus determining the lignin content. Hemicellulose was found by difference across (4).

$$\%Hemicellulose = \%Holocellulose - \%Cellulose \quad (4)$$

With elemental analysis, the content of C, H, N, and S, was determined following the regulations described in the BS EN 15104:2011[18] standard. In the proximal analysis, the amount of volatile material, ash, and fixed carbon was determined, as indicated in the BS EN 15148:2009[19] and BS EN 14775:2009[20] standards.

Sample pretreatment

Acid hydrolysis was carried out according to the methodology described by An et al.[21] with slight modifications, using a 2^3 factorial design for this purpose (Table 1). The residue sample was placed in a 500 mL batch reactor with magnetic stirring, with a 2 and 3% (v/v) HCl solution and brought to a temperature of 50 and 75 °C for 25 and 30 minutes, using a solid-liquid ratio of 1:10 (w:v). After pretreatment, the reaction mixture was cooled and subsequently separated by vacuum filtration.

Table 1. *Design of experiments for acid hydrolysis*

Concentration (%, v/v)	Reaction time (min)	Temperature (°C)
2	25	50 75
	30	50 75
3	25	50 75
	30	50 75

Lactic batch fermentation

Lactic acid fermentation was carried out as described by Bustamante et al.[22] and Vishnu et al.[23], using a 2^3 factorial design (Table 2). Lyophilized strains were activated by MRS agar medium and incubated at 37 °C for 24 h under static microaerobic conditions. Two volumes of 100 and 500 µL were taken, inoculated in 50 mL tubes with 10 mL of peptone water enriched with sucrose at 20% (w/v) under microaerobic conditions at 37 °C and 12 hours so that the bacteria adapt to the environment and its transport. The hydrolyzate obtained was adjusted to a pH between 5.5 - 6.5, and powdered sucrose was incorporated until reaching a sugar concentration of 20% (w/v) for lactic acid bacteria. The volume of the inoculum ranged from 3 to 10% (v/v) about the hydrolyzate. It was hermetically sealed, achieving an anaerobic medium at temperatures of 37 and 40 °C for 24 and 48 hours.

Table 2. *Design of experiments for lactic acid fermentation*

Microorganism volume (µL)	Fermentation time (h)	Temperature (°C)
100	24	37
		40
	48	37
		40
500	24	37
		40
	48	37
		40

Lactic acid determination

The identification and quantification of LA were performed using a Thermo Fisher Scientific ACCEL liquid chromatograph equipped with a C18 reverse phase column (5µm; 4.6x100mm) and a UV-VIS photodiode array (PDA) detector[24]. A five-point calibration curve was constructed over a range of 0.5 g/L to 2.5 g/L with LA standard. The fermentation solution was centrifuged at 4000 rpm for 25 minutes and vacuum filtered. Microfiltration was performed with a hydrophilic nylon membrane (Biomed Scientific), 13 mm in diameter and 0.22 µm in pores. The binary mobile phase consisted of a solution of acetonitrile and $3x10^{-2}$ M of H_3PO_4, with

a constant flow of 1 mL/min, in a ratio of 12:88 (v:v), respectively. Organic acid detection was performed at 210 nm with an injection volume of 10 µL, 40 °C column temperature, and a total run time of 3 minutes.

Lactic fermentation kinetics

The kinetics of LA production was calculated with the amount of lactic acid, the concentration of sugar, and biomass. The amount of total reducing sugars (TRS) was determined using the 3,5-dinitrosalicylic acid (DNS) methodology described by Monroy et al[25]. 0.5 ml of DNS reagent was added to a 0.5 ml volume of hydrolyzed corn residue sample at pH 4-4.5. The reaction was carried out in a thermostatic bath, heating to boiling for 5 minutes, and then was cooled. 5 mL of distilled water was added to each sample and stirred, and then the absorbance was determined in the spectrophotometer at 540 nm.

The amount of biomass was determined by gravimetry[26]. The residue samples were centrifuged at 4000 rpm for 20 minutes at 4°C. They were washed twice and dried in an oven at 60°C until constant weight.

Mathematical models for LA production

The kinetics of product formation was based on the application of three unstructured models: Luedeking-Piret[27], which depends on the growth rate and the instantaneous concentration of biomass (5), the logistic equation[28] that describes the production of LA (6), and the simple production model[29] that represents the generation of a product to be estimated (7).

$$\frac{dp}{dt} = \alpha \frac{dX}{dt} + \beta X \quad (5)$$

$$\frac{dp}{dt} = P_0 \left(1 - \frac{P}{P_{max}}\right) P \quad (6)$$

$$\frac{dp}{dt} = \alpha \frac{dX}{dt} \quad (7)$$

Where P is the product concentration (g/L), α dimensionless constant, β production constant (1/h), X biomass concentration (g/L), P_0 is a growth constant (1/h), P_{max} is the maximum product concentration (g/L).

Mathematical models for substrate consumption

The kinetics of substrate consumption was established by the use of different models, which are described below: Monod[30], which in general is characterized by reaching a degree of adjustment close to the experimental data (8), Luedeking-Piret[27] expressed as a simple model (9) and Hanson-Tsao[31], which is a mathematical model proposed to describe substrate consumption through biomass concentration (10).

$$\frac{dS}{dt} = \frac{-1}{Y^{X/S}} \frac{dX}{dt} \quad 8)$$

$$\frac{dS}{dt} = \frac{-1}{Y^{X/S}} \frac{dX}{dt} + qX \quad (9)$$

$$\frac{-dS}{dt} = K_s SX \quad (10)$$

Where S is the substrate concentration (g/L), $Y^{X/S}$ represents the yield (g biomass/g substrate), q is the metabolic coefficient (1/h), and K_S is the substrate saturation constant (L /g.h or 1/h).

Mathematical models for bacterial growth

Microbial kinetics was performed using the following models: Gompertz[28], which provides reliable information on the stability of a specific microorganism under certain conditions (11), logistic[32], which is an alternative for the study of the population growth of microorganisms (12), and Amrane-Prigent[32], which is characterized by its simplicity and easy convergence of the experimental data with the calculated parameters when the initial iteration values are correct (13).

$$\frac{dX}{dt} = KXLn\left(\frac{A}{X}\right) \quad (11)$$

$$\frac{dX}{dt} = \mu X \left(1 - \frac{X}{X_{max}}\right) \quad (12)$$

$$\frac{dX}{dt} = \mu_{max} \frac{1}{1 + \frac{ce^{dt}}{\mu_{max} - c}} \quad (13)$$

Where K establishes the intrinsic growth rate, A is the microbial count when time decreases indefinitely (g/L), µ is the specific growth rate (1/h), X_{max} is the maximum biomass concentration (g/L), μ_{max} represents the maximum specific growth rate (1/h), c and d are constant coefficients of the Amrane-Prigent equation (1/h), t is time (h).

Statistic analysis

The design of experiments and their respective statistical analysis was carried out with the Minitab® version 20.3 software. For the estimation of the parameters of the kinetic model, non-linear least squares regression was used using the Statistica version 10.0 software. The root means square error (RMSE) and the Akaike index (AIC) was used to determine the existence of statistically significant differences between the models used.

RESULTS AND DISCUSSION

Physical-chemical characterization of the waste

The results obtained in the characterization of the corn stover (Table 3) are compared with what other authors indicate in previous investigations, where the same residue is used.

Table 3. *Physical-chemical characterization of the waste*

Variable	Result ± σ	Variable	Result
Moisture (%)	17.97 ± 0.80	Ash % (p/p)	4.79
pH	7.49 ± 0.13	Volatile material % (p/p)	76.75
Extratable (%)	10.61 ± 0.13	Fixed carbon % (p/p)	18.46
Cellulose (%)	67.50 ± 0.70	Carbon % (p/p)	40.60
Holocelullose (%)	81.87 ± 0.40	Nitrogen % (p/p)	0.57
Lignine (%)	18.85 ± 0.41	Hydrogen % (p/p)	7.09
Hemicelullose (%)	14.37 ± 0.94	Sulfur % (p/p)	0.00
		Oxygen % (p/p)	51.74

Oyedeji et al.[33] report a moisture content on a dry basis of 15.9%, while Angelovič et al.[34] obtained an average moisture percentage of 13.6%. For the pH parameter, Gómora et al.[35] recorded 6.77 + 0.053, while Rendón[36] reported a pH of 6.76. In the characterization of the content of extractable material, values of 10.9%[37] and 12.20 + 0.41%[38] are reported. The existing differences in humidity and pH concerning other investigations are due to factors related to air humidity, temperature, age of the plant, species, characteristics, and composition present in the soil where the residue is[39]. The variation in extractable material content depends on the concentration and type of solvent used to remove the extractives[40].

In the elemental and proximal characterization, similar results were obtained to those shown in previous investigations where corn stover was used. In a similar investigation, Castro et al.[41] obtained an ash content of 6.8%, volatile material of 89.89%, and fixed carbon of 2.57%; while in the elemental analysis it obtained content of C 40.60%, N 0.8%, H 5.58%, S 0.1%, and O 39.99%. Similarly, Borja[42] reported ash of 1.73%, volatile material 79.36%, fixed carbon 17.14% and in the elemental analysis it recorded C 48.50%, N 0.4%, H 5.64%, S 0.01% and O 45.46%. The biomass of herbaceous origin presents a variable composition that depends on the type of fertilizers used during planting, the characteristics of the soil for cultivation, and other elements of nature[43].

The lignocellulosic composition obtained was compared with previous works where the value of cellulose was 51%[44,45], hemicellulose 20%[46], holocellulose 64.14% - 83.90%, and lignin 16.33% - 21.05%[35,47]. The variation, especially in the content of cellulose and holocellulose, could be associated with the differences that exist due to the type of species, plant section that can be the leaves, stem, root, and other aspects involved in plant development such as climate, nutrients, water, among others[48].

Sample pretreatment

The results obtained in the acid hydrolysis carried out as a pretreatment to the corn residue (Table 4) were analyzed through an ANOVA (Table 5) to evaluate the effect of the acid concentration (C), reaction time (t), and temperature (T) in obtaining TRS.

Table 4. *Corn stubble pretreatment results*

Acid concentration (%)	Reaction time (min)	Temperature (°C)	TRS concentration ± σ (g/L)
2	25	50	0.20 ± 0.01
		75	0.71 ± 0.09
	30	50	0.21 ± 0.03
		75	0.92 ± 0.04
3	25	50	0.67 ± 0.02
		75	0.72 ± 0.05
	30	50	0.59 ± 0.01
		75	0.70 ± 0.06

Table 5. *ANOVA results*

Variable	Effect	SC Ajust	MC Ajust	F-value	P-value
C	0.16083	0.15520	0.155204	72.61	0.000000
T	0.34417	0.71070	0.710704	332.49	0.000000
t	0.02750	0.00454	0.004537	2.12	0.164466
C*T*t	-0.03583	0.00770	0.007704	3.60	0.075819

The best hydrolysis results were obtained when working with a concentration of 2% HCl, 30 minutes, and 70 °C, with a TRS production of 0.92 g/L. These results are much lower than 21.9 g/L[49] and 24.96 g/L[50], which are values reported in other investigations where corn stover was worked with acid hydrolysis, a reaction time of 40-60 minutes, and a temperature of 120-122°C, respectively. The differences between the results obtained are attributed to factors arising from hydrolysis. Among them is the nature of the substrate, the type of pretreatment to which it has been subjected, and the conditions applied, mainly pH, temperature, and time[51].

According to the ANOVA performed, the most influential variable in obtaining TRS is temperature followed by acid concentration, as indicated by the value of F, since a sufficiently large F value indicates that the term or model is significant. In the same way, it happened when comparing the p-value with the α significance level of 0.05, resulting in only these variables having a significant effect on the applied treatment.

Batch fermentation

The initial concentration of TRS in the different working conditions ranged from 0.87 g/L to 0.80 g/L and gradually increased after the first 24 hours due to the conversion of glucose into LA. After 48 hours, much of the substrate was almost completely depleted, reaching a concentration of 0.02 g/L. This pattern of consumption throughout the experimentation was repeated, even after the variation in temperature and volume of the microorganism (Figure 1).

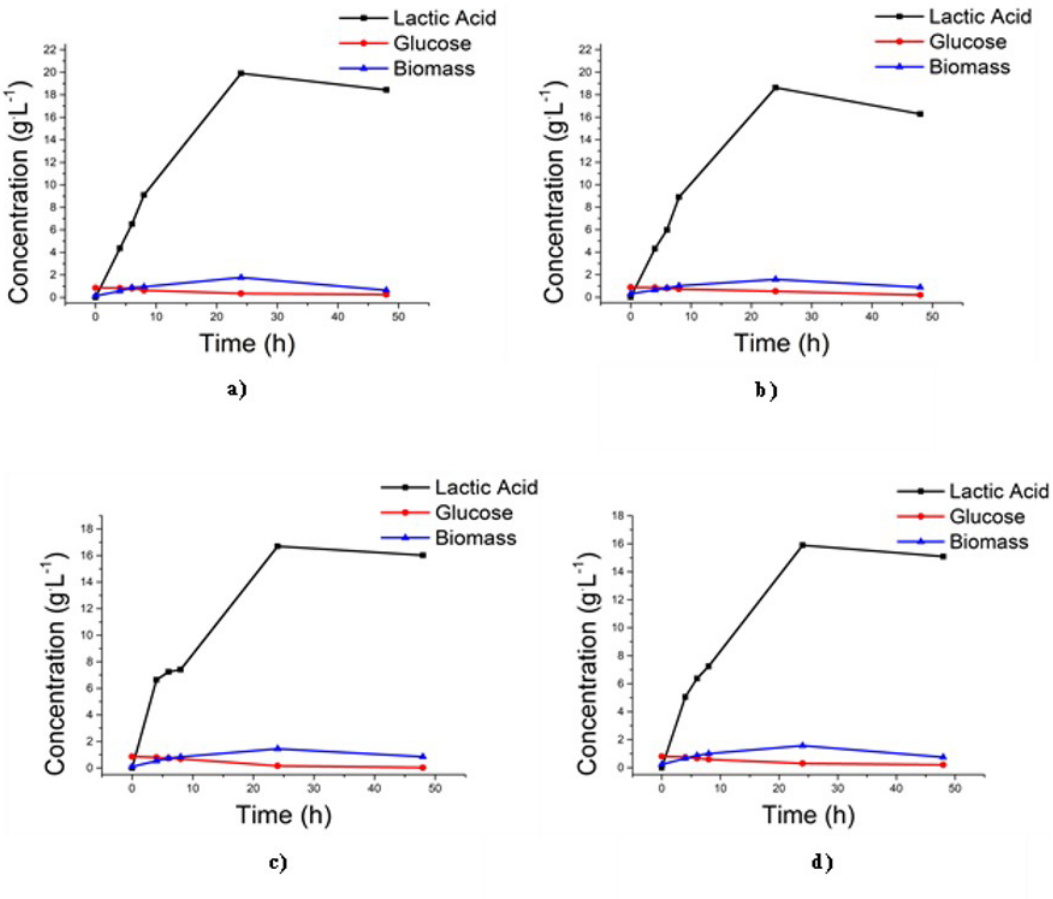


Figure 1. Batch fermentation profiles: (a) 37°C-100 µL, (b) 37°C-500 µL, (c) 40°C-100 µL, (d) 40°C-500 µL

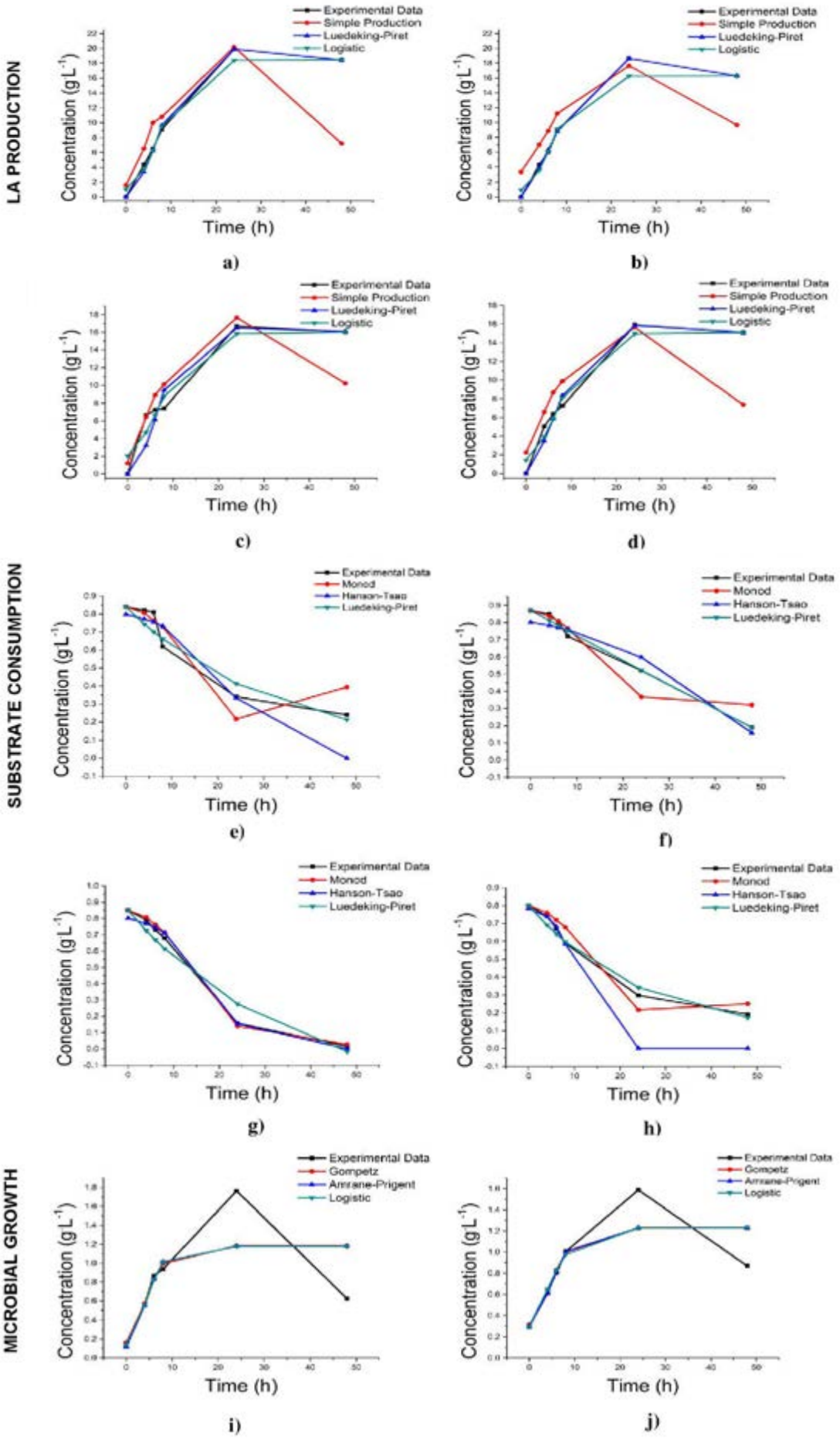
The most suitable working conditions were obtained with 37°C and a volume of 100 µL, in a fermentation time of 24 h, reaching a concentration of 19.91 g/L for LA and 1.76 g/L in the case of bacteria, which would be in its stationary phase. At 48 hours of fermentation, the yield drops to 18.48 g/L for LA and 0.63 g/L for the microorganism that is in its death phase. When working at 40°C, LA concentrations are reduced 48 hours after fermentation. The microorganism has a behavior similar to that of 37°C, where its latency phase begins in its first hours, reaching a maximum concentration at 24 hours and decreasing at 48 hours. The least influential variable in this process is the volume of the microorganism since it does not present any significant change in the different tests.

In a study where corn straw silage was used, under mesophilic temperature conditions (35°C), a set of lactic acid bacteria such as *Lactobacillus bulgaricus, Streptococcus thermophilus, Lactobacillus acidophilus, Lactobacillus plantarum, and Lactobacillus casei* as inoculum, and six days of fermentation, reached a maximum production of 10.14 $\pm$ 0.69 g/L[52]. In another investigation where corn stover was used, the *P. acidilactici* strain as a fermentative microorganism, a temperature of 42°C and 72 hours of fermentation, reached a concentration of 130.3 g/L[53].

The existing differences in the production of LA reported in other investigations may be due to factors such as the type of pretreatment of the residue, and the use of enzymes such as cellulase, which breaks down cellulose and other related polysaccharides, into multiple glucose monomers. Another aspect to take into account is the detoxification of the biomass, which eliminates inhibitory compounds generated in the pretreatment, preventing microbial growth, and the use of another fermenting microorganism or the fermentation mode to increase the LA yield at the time of fermentation[54,55].

Mathematical modeling to determine the kinetics

The kinetic data of LA production, substrate consumption, and microbial growth are represented for the three mathematical models defined in different working conditions (Figure 2). Table 6 shows the statistical parameters used to fit the batch fermentation curves. The data corresponding to LA production and glucose consumption adjust the proposed models. Unlike biomass growth, whose data are not adjusted at the proposed models in the death phase of the bacteria. The results indicate what happens during fermentation and allow the identification of anomalies in the process.


LA PRODUCTION
SUBSTRATE CONSUMPTION
MICROBIAL GROWTH
Experimental Data
Simple Production
Luedeking-Piret
Logistic
Monod
Hanson-Tsao
Gompetz
Amrane-Prigent
Concentration (g L-1)
Time (h)
a)
b)
c)
d)
e)
f)
g)
h)
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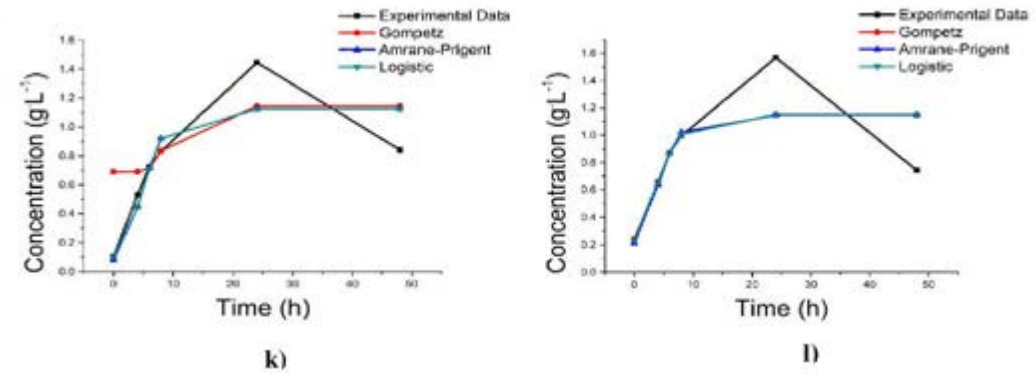

Figure 2. *Fermentation kinetic modeling profiles: (a) 37°C-100 µL, (b) 37°C-500 µL, (c) 40°C-100 µL, (d) 40°C-500 µL, (e) 37°C-100 µL, (f) 37°C-500 µL, (g) 40°C-100 µL, (h) 40°C-500 µL, (i) 37°C-100 µL, (j) 37°C-500 µL, (k) 40°C-100 µL, (l) 40°C-500 µL*

Table 7. *Statistical parameters of mathematical models for batch fermentation*

Conditions		Model	Adjusted coefficients	R^2	$\bar{R}^2$	RMSE	AIC
Acid lactic production	a	Simple production	α: 11.468	0.528	0.410	5.441	10.353
		Luedeking-Piret	α: 13.048 - β: -0.021 - Kp: 0.309	0.996	0.990	0.654	1.978
		Logistic	α: 16.768 - β: 0.361	0.987	0.978	1.007	2.980
	b	Simple production	α: 11.096	0.701	0.627	3.925	8.652
		Luedeking-Piret	α: 16.876 - β: -0.087 - Kp: 0.198	0.999	0.997	0.305	-2.001
		Logistic	α: 16.448 - β: 0.380	0.972	0.954	1.335	4.451
	c	Simple production	α: 12.179	0.771	0.714	3.032	7.306
		Luedeking-Piret	α: 12.474 - β: -0.012 - Kp: 0.374	0.924	0.811	2.250	8.420
		Logistic	α: 7.023 - β: 0.268	0.947	0.911	1.636	5.509
	d	Simple production	α: 9.966	0.580	0.475	3.976	8.719
		Luedeking-Piret	α: 12.494 - β: -0.026 - Kp: 0.259	0.979	0.948	1.143	4.891
		Logistic	α: 9.444 - β: 0.299	0.972	0.954	1.141	3.630
Glucose consumption	e	Monod	qs: 0.015	0.847	0.809	0.103	-10.317
		Luedeking-Piret	µm: -0.033 - m: 0.218	0.928	0.880	0.091	-10.273
		Handson-Tsao	Ks: -0.999 - µ: 0.120	0.776	0.627	0.139	-7.329
	f	Monod	qs: 0.013	0.877	0.846	0.092	-10.910
		Luedeking-Piret	µm: -0.002 - m: 0.051	0.991	0.986	0.027	-15.879
		Handson-Tsao	Ks: -0.748 - µ: 0.063	0.945	0.864	0.068	-11.032
	g	Monod	qs: 0.020	0.995	0.989	0.024	-39.850
		Luedeking-Piret	µm: -0.003 -m: 0.359	0.958	0.930	0.082	-10.099
		Handson-Tsao	Ks: -1.567 - : 0.141	0.993	0.989	0.033	-14.854
	h	Monod	qs: 0.015	0.931	0.914	0.065	-12.738
		Luedeking-Piret	µm: -0.043 - m: 0.173	0.982	0.970	0.037	-14.260
		Handson-Tsao	Ks: -0.247 - µ: 0.339	0.587	0.311	0.178	-6.055
Biomass growth	i	Gompetz	C: 1.045 - B: 0.370 - M: 3.657	0.556	-0.111	0.468	0.234
		Amrane- Prigent	µm: 0.564 - d: 0.476 - c: 0.254	0.562	-0.096	0.465	0.198
		Logistic	K: 1.181 - r: 0.474	0.555	0.258	0.406	-1.76
	j	Gompetz	C: 0.929 - B: 0.341 - M: 4.292	0.718	0.294	0.295	-2.162
		Amrane- Prigent	µm: 0.195 - d: 0.501 - c: 0.014	0.720	0.299	0.294	-2.179
		Logistic	K: 1.232 - r: 0.309	0.716	0.527	0.256	-4.148
	k	Gompetz	C: 0.455 - B: 0.077 - M: 8.269	0.421	-0.446	0.432	-0.179
		Amrane- Prigent	µm: 0.632 - d: 0.449 - c: 0.0308	0.791	0.477	0.259	-2.829
		Logistic	K: 1.125 - r: 0.478	0.791	0.652	0.225	-4.836
	l	Gompetz	C: 0.929 - B: 0.388 - M: 3.349	0.645	0.112	0.339	-1.437
		Amrane- Prigent	µm: 0.369 - d: 0.489 - c: 0.119	0.644	0.406	0.339	-1.431
		Logistic	K: 1.151 - r: 0.415	0.645	0.409	0.294	-3.442

RMSE: Mean square error, AIC: Akaike Index

It is observed that the Luedeking-Piret model is the one that best fits the kinetics of LA production, with a coefficient of determination (R^2) close to 1, a mean square error, and a lower Akaike index than the rest of the models[56]. The RMSE is a precision measure that indicates how the model predicts the response, being an important criterion in these cases[56]. Similarly, the AIC provides a simple and objective method, which selects the most appropriate model to characterize the experimental data[57]. These results coincide with the values reported in previous works, where the Luedeking-Piret kinetic model is selected as the one that best represents the experimental data[58,59].

For substrate consumption, the Monod and Luedeking-Piret models are the ones that exhibit a closer fit to the experimental data, the latter being the one that best fits if the values of R^2, AIC, and RMSE are considered. The experimental data resemble data reported in previous studies, where the data predicted with the model were highly compatible with the experimental kinetics, with satisfactory R^2 and RSME[60,61].

Finally, for the growth of the microorganism, the best fit with lower AIC and RMSE values is presented with the logistic model. However, the correlation coefficient allows us to establish that the experimental data in the death phase do not adequately fit the model with R^2 close to 0.70. In other investigations this model was also selected as the one with the best adaptation but with limitations in the stationary phase and cell death[62,63]. The adjustment of the microbial growth models, as in the case of the logistic model, is influenced by the phases where the latency, growth, and stationary state are described but not death[64].

CONCLUSIONS

The possibility of using corn stover as a substrate for the production of LA was demonstrated, representing an alternative for the revaluation of said residue. The highest LA production and bacterial growth occurred at 24 h, with a maximum concentration of 19.91 g/L and 1.76 g/L, respectively. In the case of substrate consumption, it gradually decreased during 48h until reaching 0.02 g/L. With the experimental results and the use of equations, the kinetics of LA production was modeled. For LA production and glucose consumption, the Luedeking-Piret model had the best fit of the estimated data compared to the experimental ones. For the growth of the microorganism, the logistic model had a better fit of the data compared to the other two models. However, it did not adequately represent the data in the death phase.

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